

Figure 1.

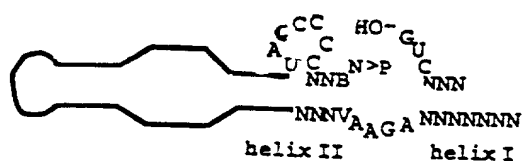
205020-35679004

Trans Cleavage and Ligation

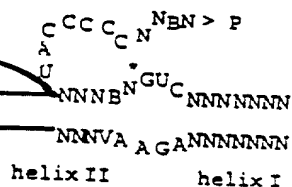
T7 promoter

Library Sequences

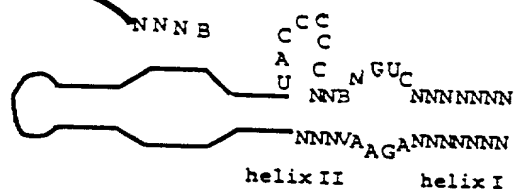
- Transcribe library with T7 RNA polymerase.
- Cis-cleavage occurs.
- Purify those randomized ribozymes that have undergone cis-cleavage.



React with target RNA in Trans



Trans Ligation

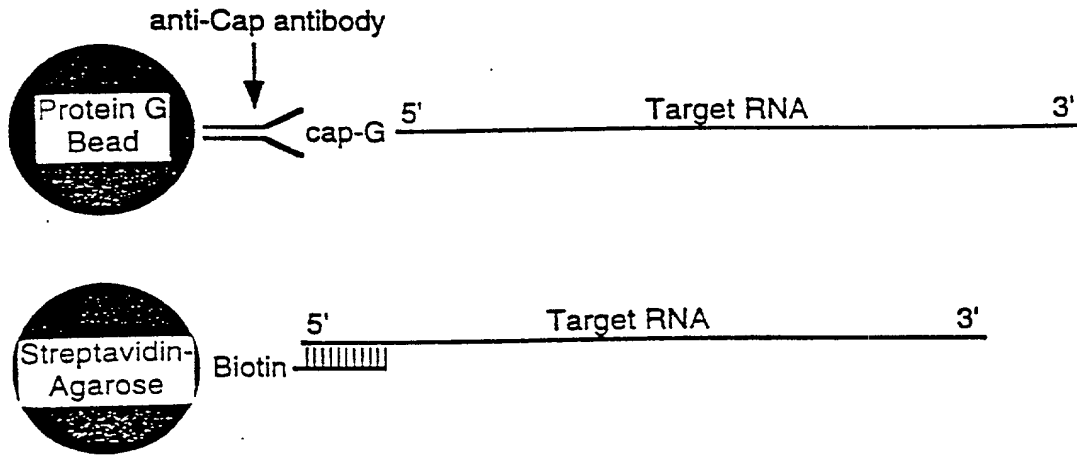


- Trans-ligated products are isolated and amplified by RT-PCR.
- Trans-ligated ribozymes can then be further amplified and subcloned into AAV vectors for production of a target specific ribozyme gene vector library.

Fig. 2

Attaching RNA target to solid support

Binding target RNA at 5' end:



Binding target RNA at 3' end:

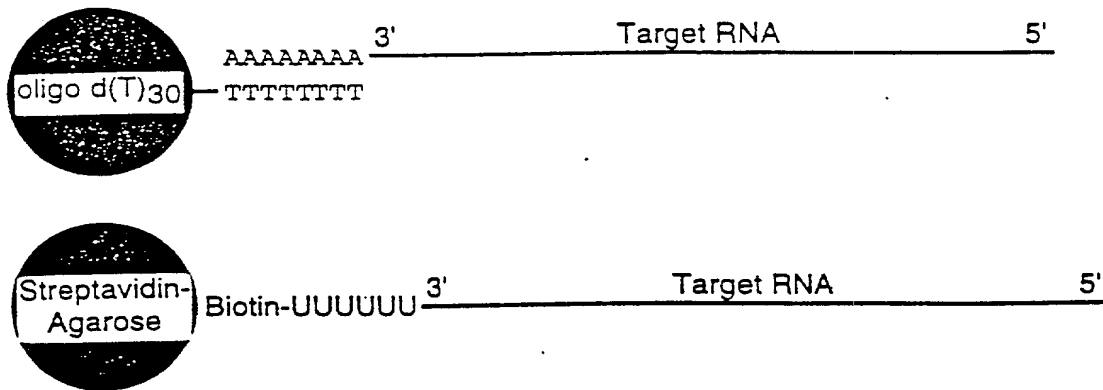
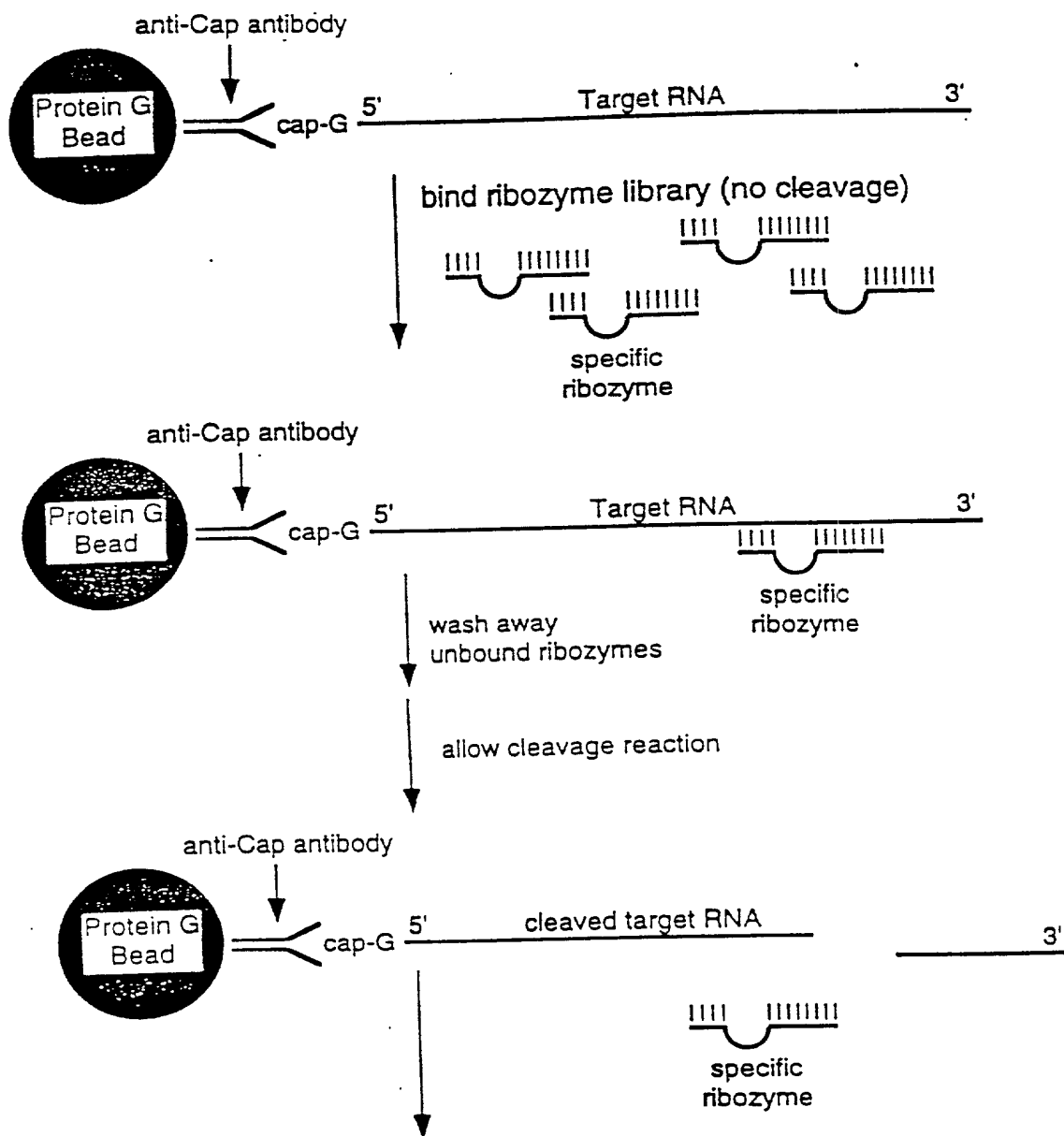


Fig. 3

In vitro selection of optimal ribozymes



Specific ribozymes elute off the solid support
 Amplify, synthesize and re-apply ribozymes to new column
 Carry out selection multiple times, as necessary

Fig. 4

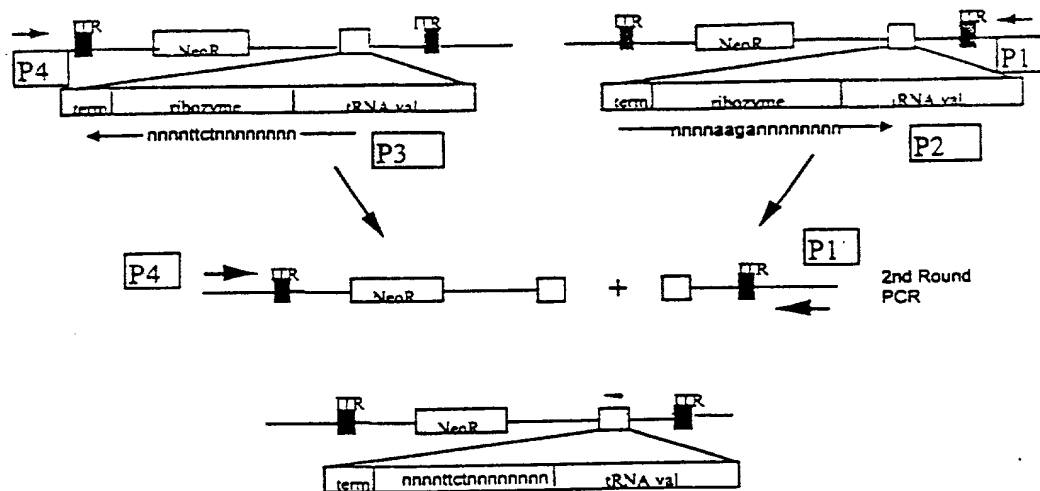


Fig. 5

Cleavage of Various Target RNAs with AAV- Ribozyme Vector Library

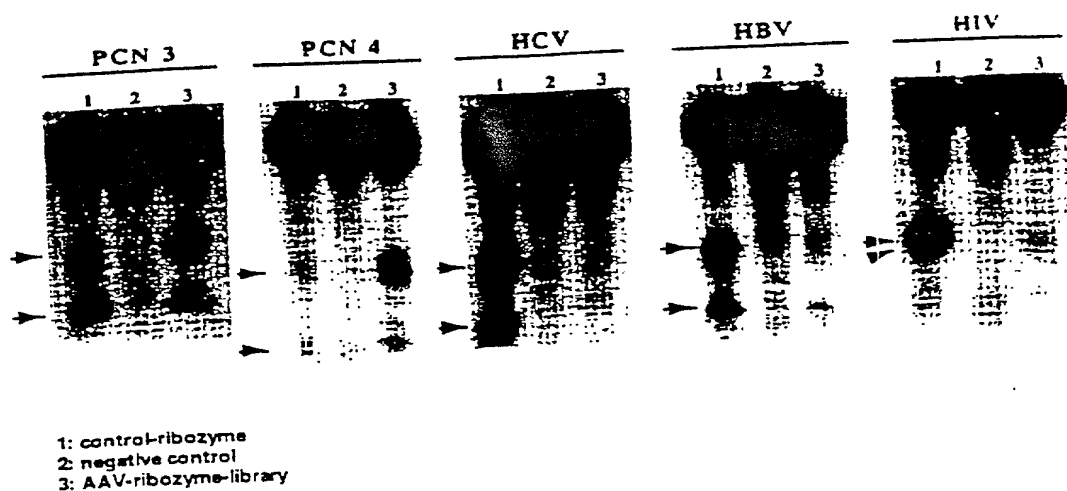


Fig. 6

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p1014/2kb

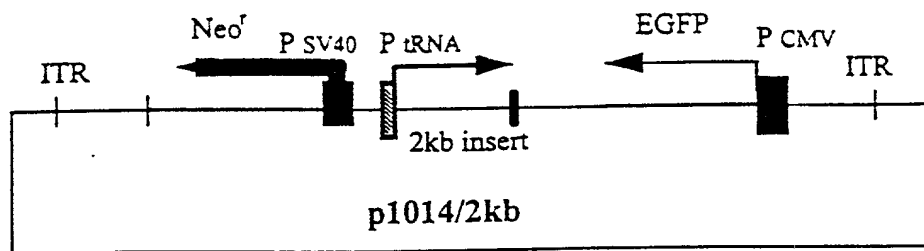


Fig. 7

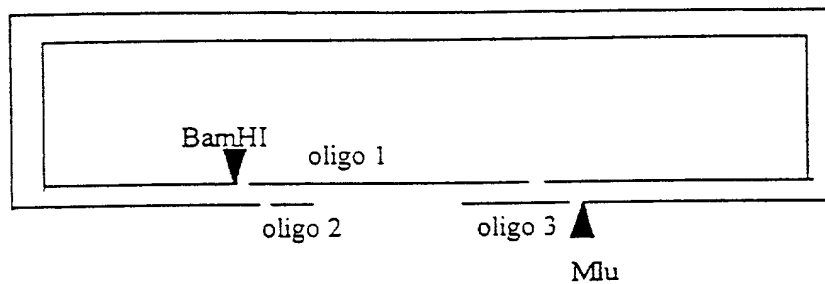


Fig. 8

pAAV-Hygro-PGK

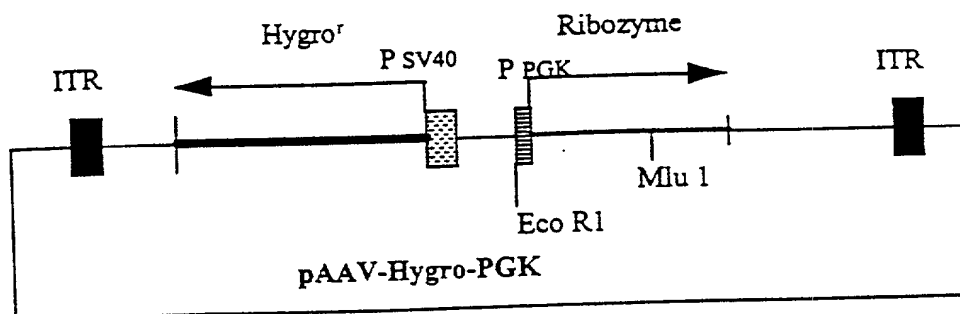


Fig. 9

pPol II/PGKmus/neo

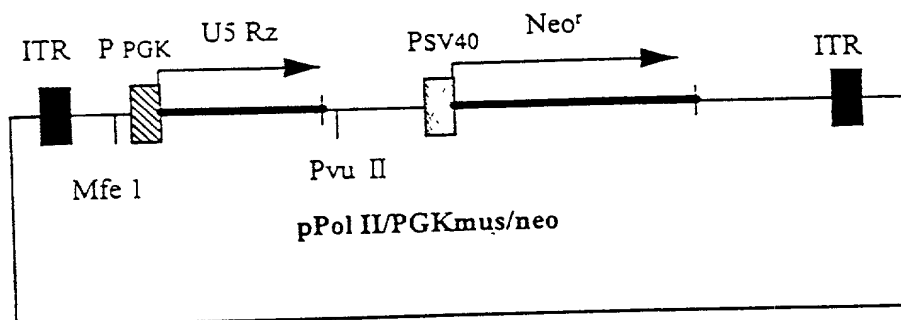


Fig. 10

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p 1015

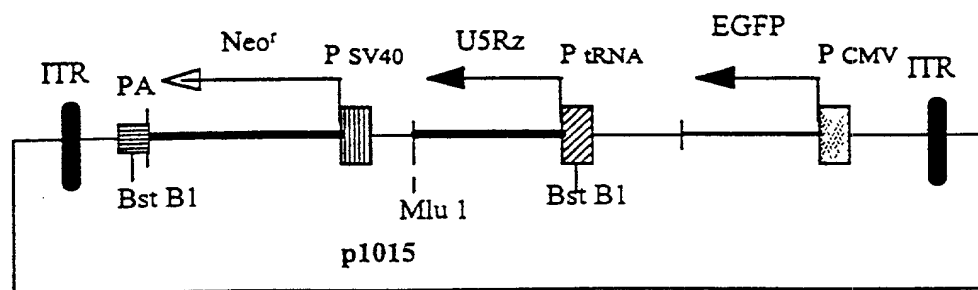
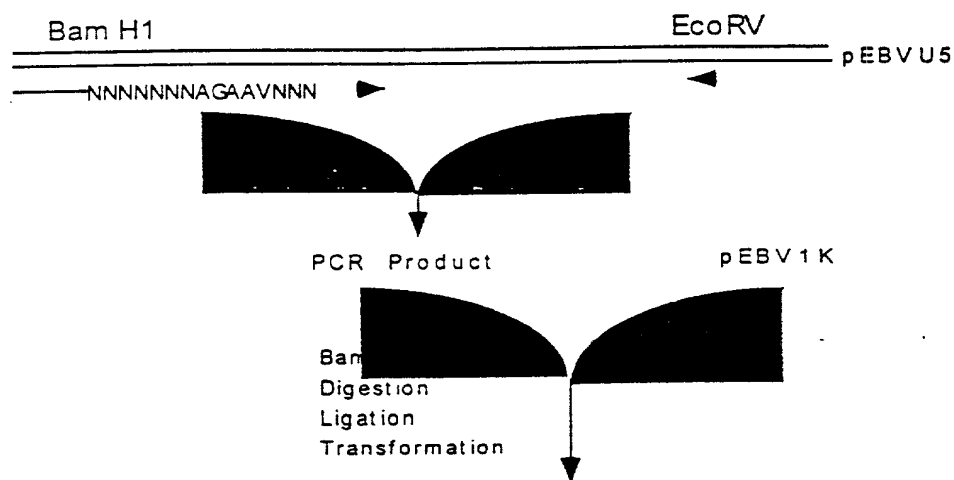


Fig. 11



ERL030398

Fig. 12

pLHPM-2kb

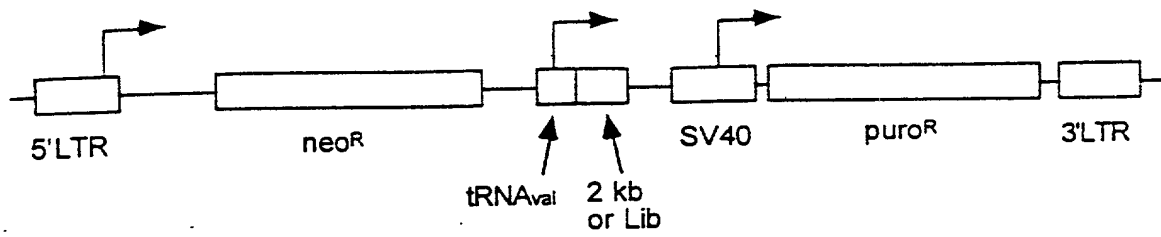


Fig. 13a

pLPR-2kb

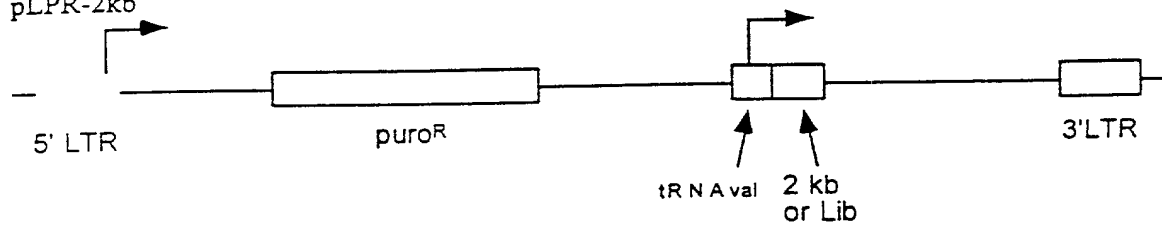


Fig. 13b

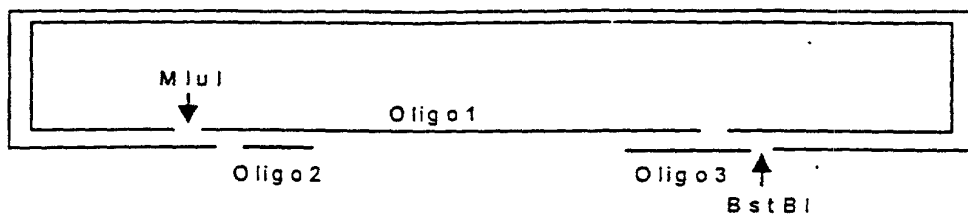


Fig. 14

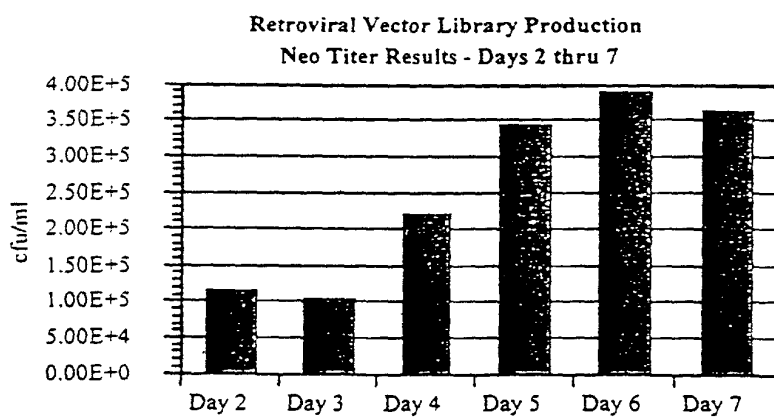


Fig. 15

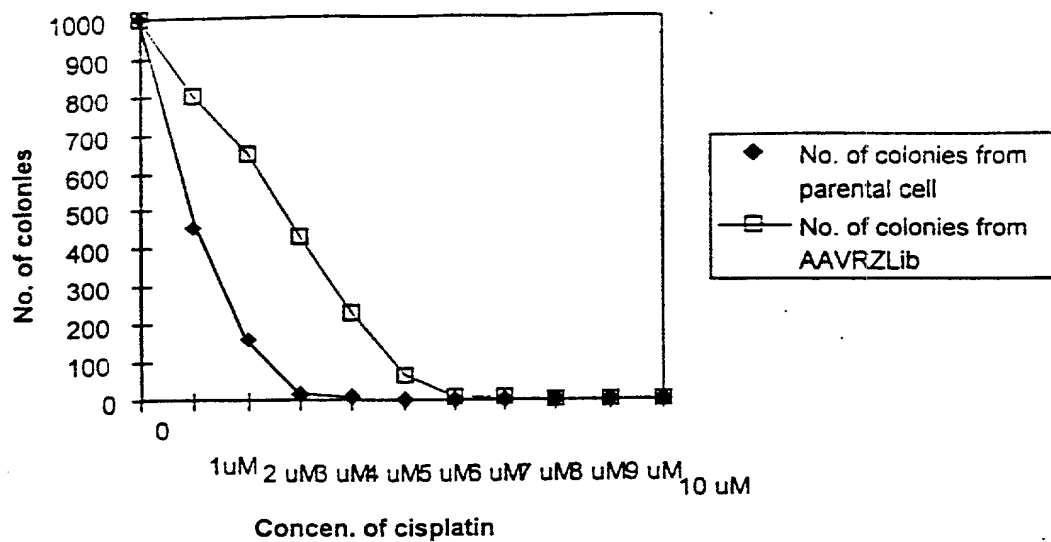


Fig. 16

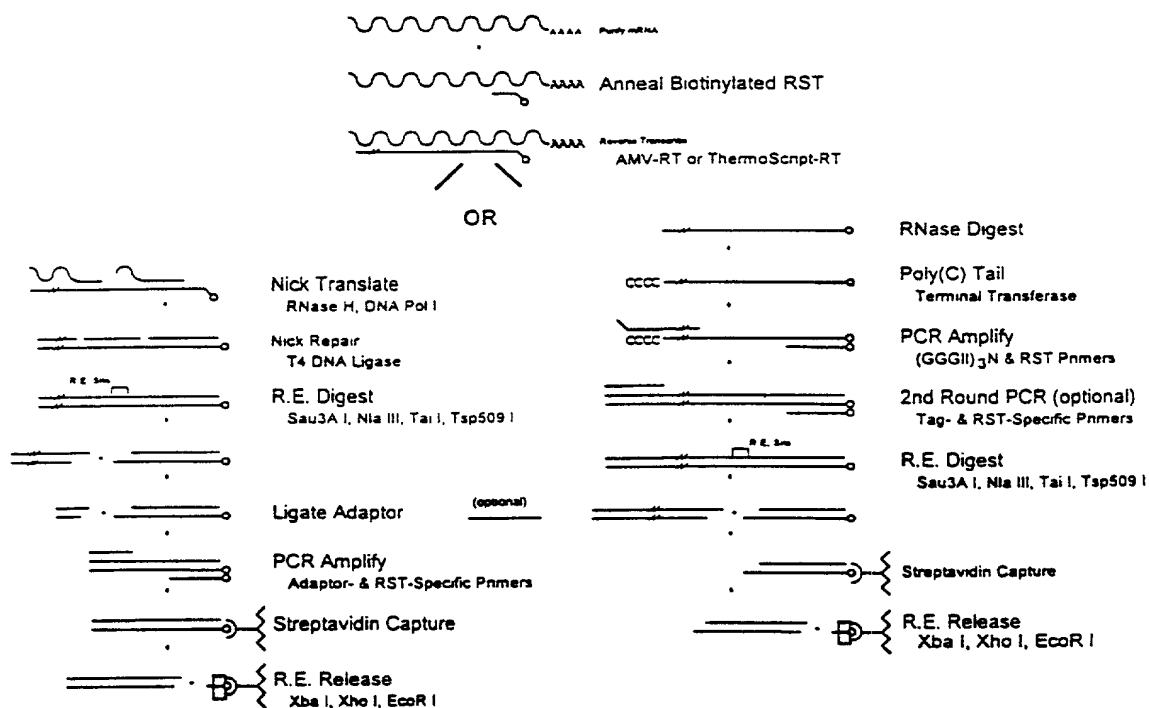


Fig. 17

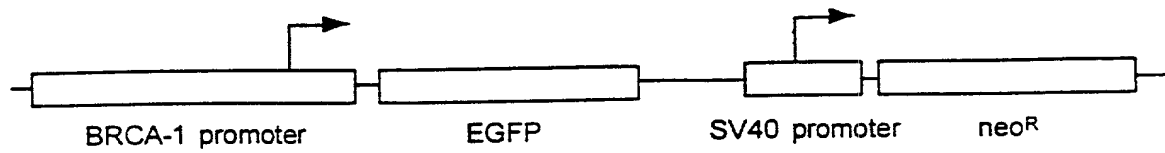


Fig. 18

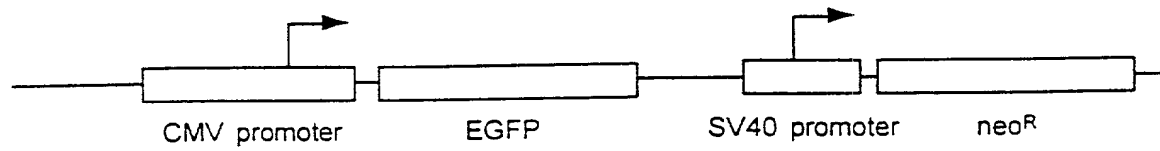


Fig. 19

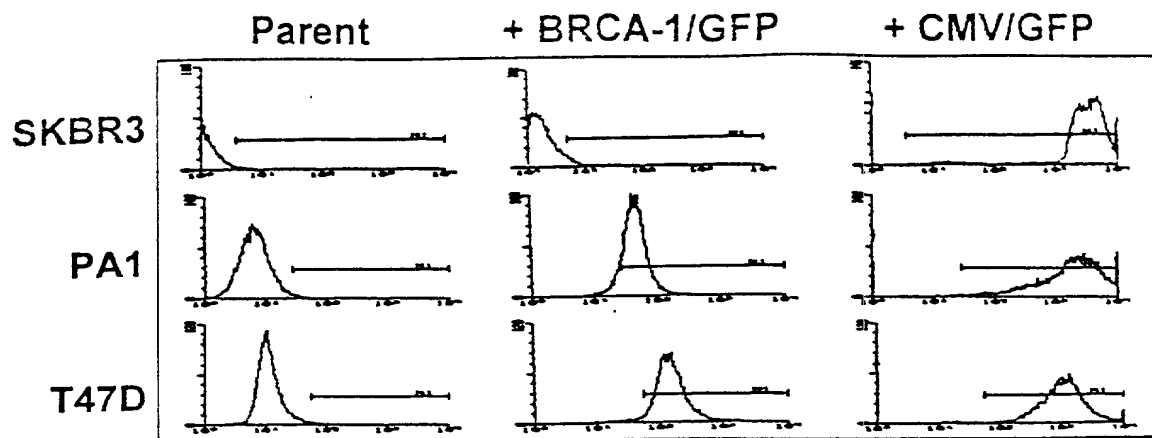


Fig. 20

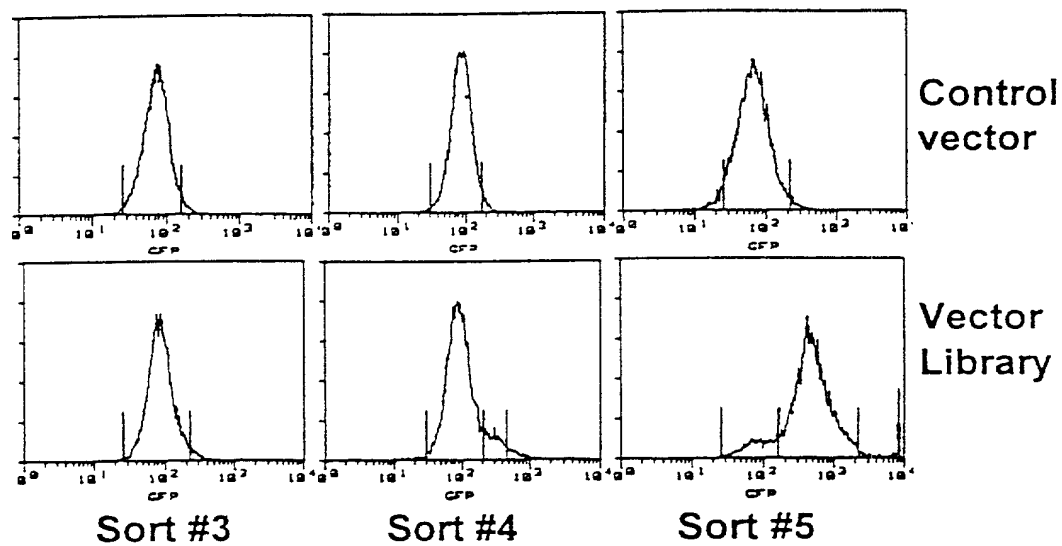


Fig. 21

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SV 40 - hygro - HCV IRES - HSV-tk

Fig. 22

Transfection (Electroporation)

HeLa

hygromycin
selection

stable cell line

SV40-hygro-IRES-tk (5' TK)

no ribozyme

no inhibition of
IRES translation

HSV-tk expression

cell death

RzLibrary

inhibition of
IRES translation

no HSV-tk

cell survival

Ganciclovir

Fig. 23

Figure 24

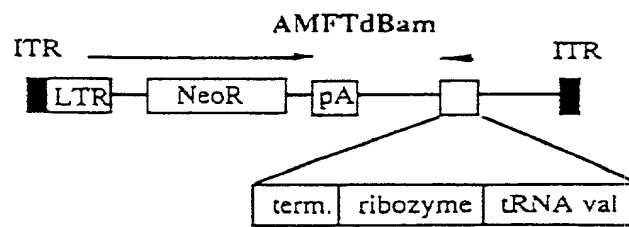


Figure 25

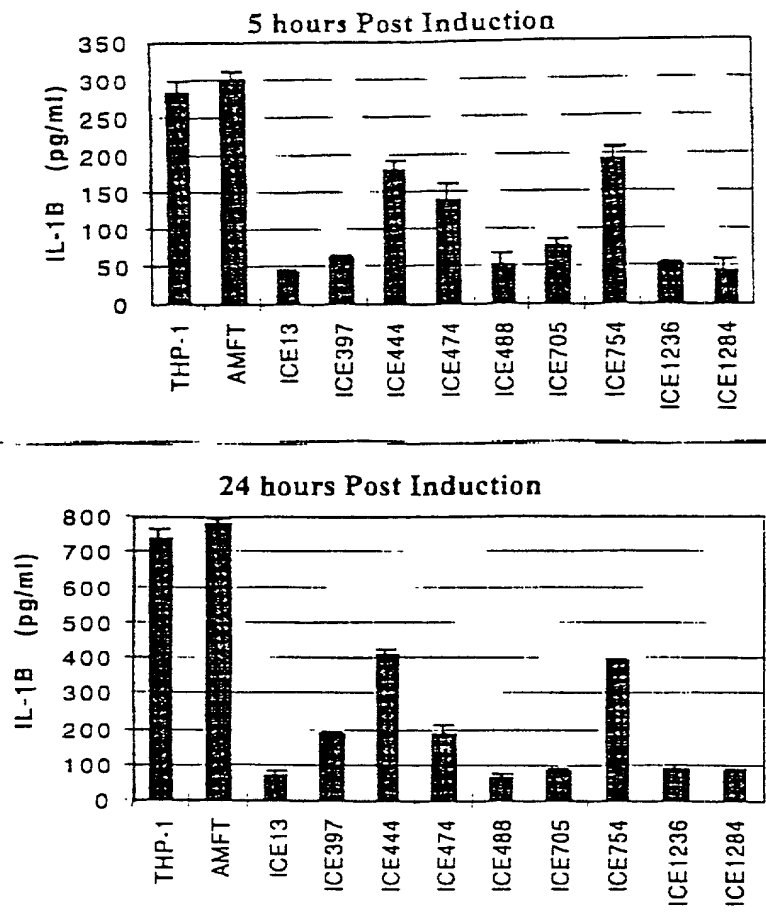
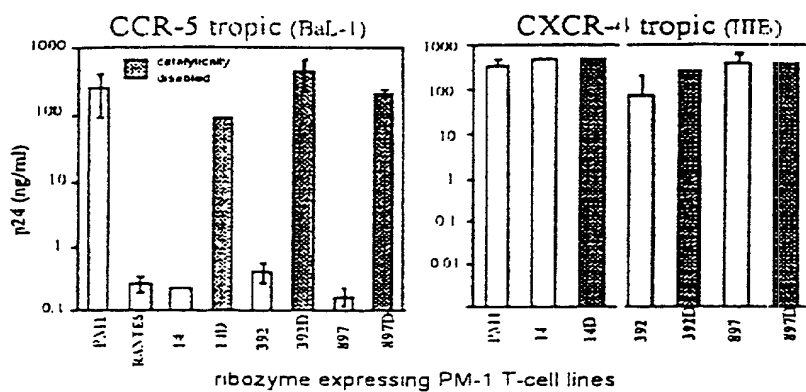


Figure 26

Resistance to CCR-5 tropic HIV



Surface expression

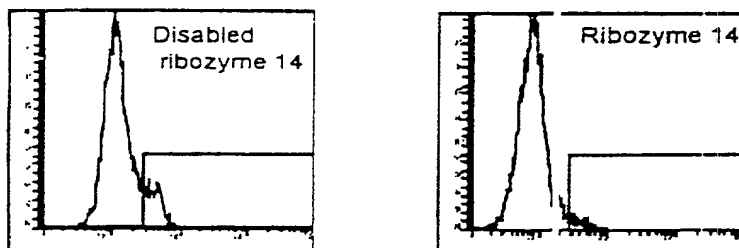
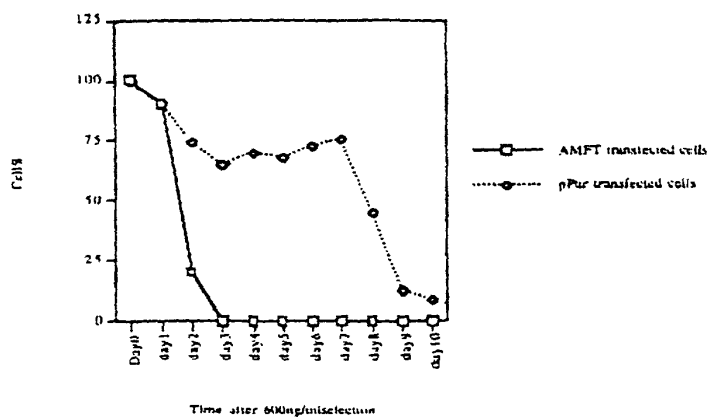


Figure 27

Puromycin selection on pPur and AMFT transfected A549 cells



Puromycin selection on pPur and AMFT transfected Hela cells

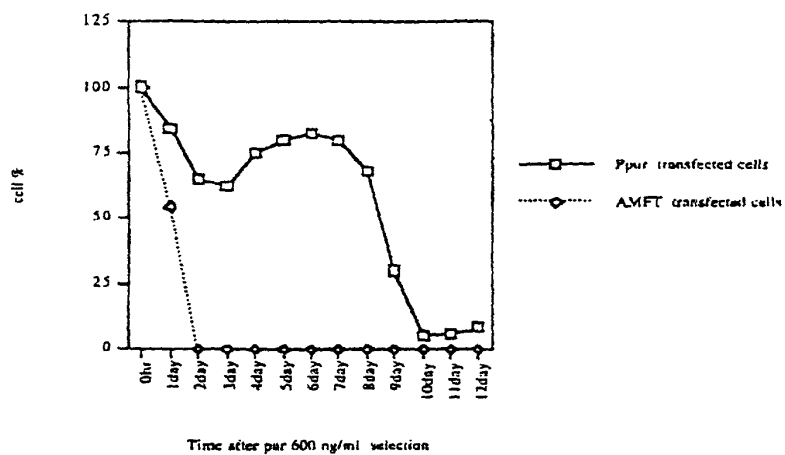


Figure 28

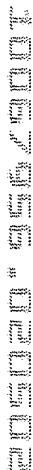


Figure 29

Auxiliary Sequences

Time Course for Cleavage by U5 - SLI1 Chimeric Ribozymes

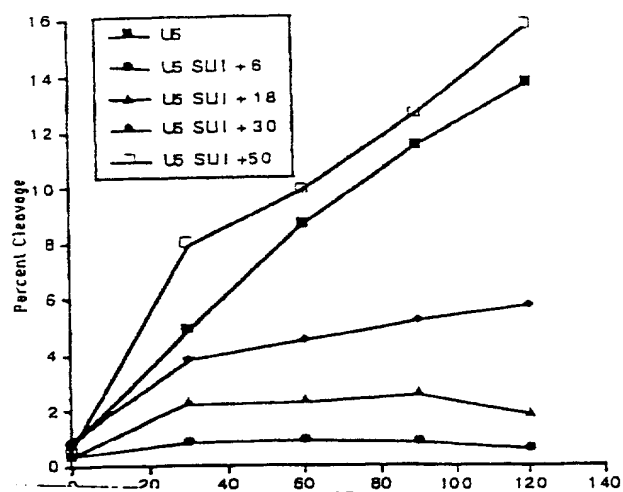
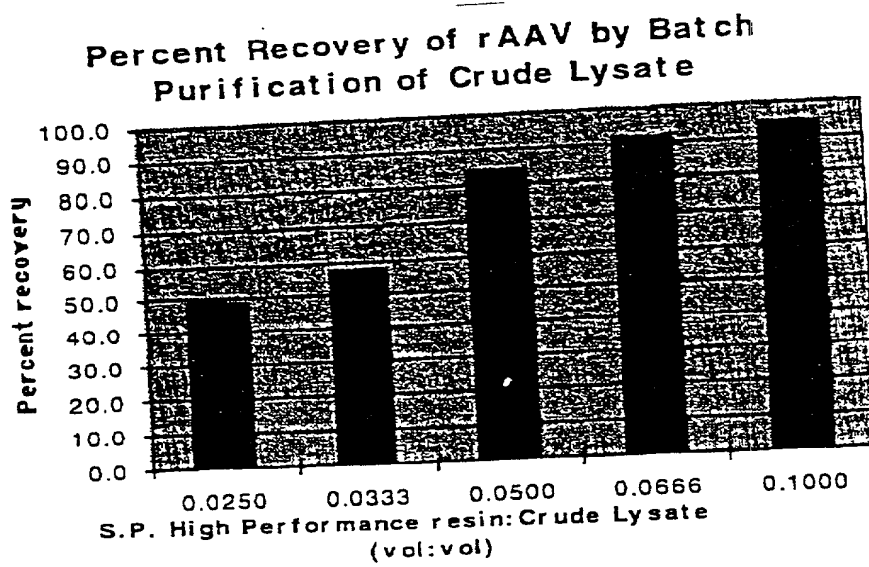


Figure 30



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